

بسم الله الرحمن الرحيم

CAPRINE BRUCELLOSIS IN OMDURMAN AREA

By

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DEDICATION

To my father

To my mother

To my Lecturers

To all my friends

*To every body sick and he is hopeless
of being healthy again*

PREFACE

This work was carried out in the Department of Preventive Medicine and Veterinary Public Health, Faculty of Veterinary Medicine, University of Khartoum, under supervision of Dr. Tawfig El Tigani Mohammed.

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SUMMARY

Caprine brucellosis (caused by *Brucella melitensis*) is a serious disease which causes economic losses in goats and a serious human illness if transmitted to man.

Serological tests on caprine brucellosis were carried out to determine the prevalence of the disease in goats in Omdurman area.

A total of 164 samples of sera and 138 samples of milk were examined.

The samples were collected from different localities in Omdurman area.

Three serological tests (RBPT) Rose Bengal Plate Test (CTAT) Capillary Tube Agglutination Test and the agar gel precipitation test were carried out.

The results showed that the rate of positive reactors was (16.57%) by RBPT and CTAT. A much lower percent of positive reactors (9.28%) was obtained with milk ring test.

خلاصة الأطروحة

الإجهاض المعدي مرض خطير يؤدي إلي تدني إنتاج الماعز بالإضافة إلي ضرره الكبير بصحة الإنسان.

تم إجراء اختبارات معملية لدراسة مرض البروسيلا في الماعز لتحديد معدل انتشار المرض في منطقة ادمرمان.

وقد تم فحص 164 عينة سيرم و 138 عينة لبن، وقد جمعت العينات من أماكن مختلفة من منطقة ادمرمان.

تم فحص العينات بواسطة ثلاثة اختبارات هي الـروزبنقال والتلاذن في الأنبوب الشعري والترسيب في الأجار. وقد فحصت عينات اللبن بواسطة اختبار حلقة اللبن.

أوضحت النتائج أن نسبة الحيوانات الايجابية كانت (75ر16%) في كل من اختباري الـروزبنقال والتلاذن في الأنبوب الشعري.

أما بالنسبة لعينات اللبن نسبة أقل من الحيوانات التي تم اختبارها قد كانت موجبة لاختبار حلقة اللبن (28ر9%).

INTRODUCTION

Brucellosis is an infectious disease of animals and man, produced by any of the members of the genus *brucella*. It is of both public health and economic importance world wide.

Brucella is a group of bacteria morphologically and antigenically similar (Bergy's, 1980).

It has six species according to the primary host. *Brucella melitensis* (*Br. metitensis*) was the first species reported as the cause of serious disease of men resulting from consumption of raw goat milk. The disease is known as brucellosis, undulant or Malta fever.

The disease in cattle is known by many names such as infectious abortion, Bang's disease, sinking of the calves and contagious abortion.

In man the disease was also known as Mediterranean fever, Malta fever, goats fever and undulant fever (Carpenter and Hubbert, 1963).

David Bruce, a British military medical officer stationed in Malta described the aetiology of the disease in man in 1984.

The bacteriologist Zammit Themistocles, a member of the Mediterranean fever commission, isolated *Br. melitensis* in 1897 from the milk of goats that had aborted. He also discovered that drinking milk from these goats was the reason for out breaks of Malta fever among British soldiers stationed in Malta.

Brucellosis is caused in cattle by *Brucella abortus* (*Br. abortus*), in sheep and goat by *Br. melitensis*, and in swine by *Brucella suis*

(*Br. suis*). *Brucella ovis* (*Br. ovis*) which causes orchitis in rams and *Brucella neotome* (*Br. neotome*) which is a pathogen of rats.

Brucella melitensis primarily affects the reproductive tract of sheep and goats, and *Br. melitensis* infection is characterized by abortion, retained placenta and, to a lesser extent, impaired fertility and sterility and decrease in milk yield due to mastitis, also hygroma, orchitis and long calving intervals (Musa *et al.*, 1990).

In Sudan animal brucellosis was suspected as early as 1904 and was first reported by Bennet (1943) in Khartoum. Subsequently many authors surveyed the disease in different animal species in different localities in Sudan (RIAS, 2004).

The Sudan has 43.8 million goats (AOAD, 1998). These animals are of great economic importance and are kept for meat, milk, hair and skin.

The rates of positive reactions in goats were 2.5 - 5.9% in the Gezira (Dafalla and Khan, 1958), 5.7 - 8.3% in the upper Nile province (Nasri, 1962), 1.5% in Wadi Halfa (Abdullah, 1966). In Khartoum different rates were reported (Elsawi *et al.*, 1981), 2.2%; (Fayza *et al.*, 1990), 1.0% (Ginawi, 1997), 0.0% . In Nyala (RIAS, 2004) 2.96%.

In recent years, the Ministry of Animal Resources paid great attention to brucellosis in the country. Many seminars and workshops were held to discuss and formulate plans for the study and control of the disease in Sudan with special emphasis on brucellosis in small ruminants.

This was considered apriority because the ministry is paying great attention to goat production and has already imported foreign breeds to improve local breeds.

Due to the small number of investigations on caprine brucellosis in the country, the present work was done in Khartoum state for the following reasons.

1. Academic purpose to provide additional information on problem.
2. Milk consumption has greatly increased in recent years in Khartoum state and part of the milk is supplied by goats. The animals are also kept in close association with man.
3. *Br. melitensis* cause a very serious disease in man and as the goat is the main source of infection, it was considered necessary to investigate the disease in goats. This is particularly obvious when we take into consideration the fact that there are now many human febrile conditions of uncertain aetiology and that testing human sera for antibodies to *brucella* has increased.
4. Khartoum state has become a major supplier of goats meats and live animals for export and some of the importing countries demand a certificate of freedom from brucellosis.

It is necessary to determine the disease situation in the state, so as to formulate a control policy and guarantee a steady supply of *brucella* - Free goats for export.

CHAPTER ONE

LITERATURE REVIEW

1.1 Brucellosis:

Brucellosis is a zoonotic contagious bacterial disease caused by members of the genus *brucella* (Corbel and Hendary, 1983).

In animals the disease is characterized by a bacteraemia followed by localization of the organism in the reticuloendothelial tissue, reproductive organs and sometimes joints. Reproductive tract lesions of the pregnant cows, sheep and goats may lead to death and expulsion of the foetus.

Brucella can also cause lesions in male reproductive tract in cattle, sheep, goats and dogs and also bursitis in the horse (Gillespie and Timorey, 1981).

Brucellosis is still a major problem, widely distributed throughout the world, mainly in developing countries due to traditional feeding habits and the failure to maintain standards of hygiene because of socioeconomic conditions (Ozekicit *et al.*, 2003).

Brucella in sheep and goats occur endemically in the Mediterranean region, specially along its northern and eastern shores, stretching through central Asia as far south as the Arabian peninsula and as far east as Mongolia. Parts of Latin America are also seriously affected, specially Mexico, Peru and northern Argentina. The disease also occurs in Africa and India. However, North America (except

Mexico) is believed to be free, as are northern Europe (except for sporadic incursions from the south), Southeast Asia, Australia and New Zealand. (FAO, OIE, WHO; 1997).

1.2 The Genus *brucella*:

1.2.1 Morphology:

Brucella are cocco bacilli or short rods 0.6 to 1.5 mm long by 0.5 to 0.7mm in width. They are arranged singly and less frequently in pairs or small groups (Evans, 1918).

The morphology of *brucella* is fairly constant except in old culture, where polymorphic forms may be evident.

Brucella are non-motile. They do not form spores, flagella or pili. True capsules are not produced.

Brucella are gram- negative and usually do not show bipolar staining. They are not truly acid- fast but resist decolouration by weak acids, thus stain red by the stamp's modification of zeihl-neelsen method, which is sometimes used for the microscopic diagnosis of brucellosis from smears of solid or liquid specimens (Cowan and steel, 1993) and (Scientific Committee on Animal Health and Animal Welfare, 2001).

1.2.2 Culture and growth characteristic:

Brucella members are aerobic, but some strains require an atmosphere containing 5-10% carbon dioxide (CO₂) added for growth, specially on primary isolation (Buxton and Fraser, 1977). The optimum growth temperature is 36-38°C but most strains can grow between 20°C and 40°C. The optimum pH for growth is near pH 6.8 (Buxton and Fraser, 1977) and (Scientific Committee on Animal Health and Animal

Welfare, 2001).

According to Bergey's Manual, of systemic bacteriology (1984), *brucella* require biotin, thiamin and nicotinamide.

The growth is improved by serum or blood. The growth of most *brucella* strains is inhibited on media containing bile salts, tellurite or selenite. Growth is usually poor in liquid media unless culture is vigorously agitated. On suitable solid media *brucella* colonies are circular and 2-4 mm in diameter.

It does not require supplementary carbon dioxide to grow and it takes 3-5 days incubation at 37°C for visible colonies to appear (Cowan and Steel, 1993).

1.2.3 Biochemistry:

The metabolism of *brucella* is oxidative and *brucella* cultures show no ability to acidify carbohydrate media in conventional tests. (Cowan and Steel, 1993).

The *brucella* species are catalase positive and usually oxidase positive and they reduce nitrate to nitrite (except *Br. ovis* and some *Br. canis* strains), the production of H₂S from sulphur containing amino-acids also varies, the indole and voges-proskauer tests are negative (Cowan and Steel, 1993).

1.2.4 Taxonomy of the genus:

Classical methods to identify *brucella* include serotyping, phage typing and oxidative metabolic tests.

Characters for classification of the genus *brucella* and biovar differentiation according to corbel (1990) and Alton *et al.* (1988) are

shown in Tables (1 and 2).

Table 1 Biovar and differentiation of the species of the genus *brucella* according to Alton *et al.* (1988):

Species	Biovar	CO ₂ require- -ment	H ₂ S product- -tion	Growth on dyes ^a		Agglutination in sera ^b		
				Thionine	Basic fuchsin	A	M	R
<i>Br. melitensis</i>	1	-	-	+	+	-	+	-
	2	-	-	+	+	+	-	-
	3	-	-	+	+	+	+	-
<i>Br. abortus</i>	1	+c	+	-	+	+	-	-
	2	+c	+	-	-	+	-	-
	3	+c	+	+	+	+	-	-
	4	+c	+	-	+d	-	+	-
	5	-	-	+	+	-	+	-
	6	-	-	+	+	+	-	-
	7	+or -	+	+	+	-	+	-
<i>Br. suis</i>	1	-	+	-	-e	+	-	-
	2	-	-	+	-	+	-	-
	3	-	-	+	+	+	-	-
	4	-	-	+	-f	+	+	-
	5	-	-	+	-	-	+	-
<i>Br. neotomae</i>		-	+	-g+	-	+	-	-
<i>Br. ovis</i>		+	-	+	-f	-	-	+
<i>Br. canis</i>		-	-	+	-f	-	-	+

a = dye concentration 20µg/ml in serum dextrose medium (1: 50000)

b = A=A mono-specific antiserum;

M= Mount specific antiserum;

R = rough *brucella* antiserum

C = usually positive on primary isolation.

d = some strains do not grow on dyes

e = some strains are resistant

f = negative for most strain

g = grow that 10µg/ml (1: 100000 thionine)

Table 2 classification of the genus *brucella* according to corbel (1990)

Proposed taxonomic Biover designation		Nomen species biover		CO ₂ Requirement	H ₂ S production	Growth on media containing	
						Thionine	Basic fuchsin 20mg/ml
<i>Br. melitensis</i> biover	1	<i>Br. melitensis</i>	1	-	-	+	+
	2		2	-	-	+	+
	3		3	-	-	+	+
<i>Br. melitensis</i> biover abortus	1		1	(+)	+	-	+
	2		2	(+)	+	-	-
	3		3*	(+)	+	+	**
	4		4	(+)	+	-	+
	5		5	-	-	+	+
	6		6*	-	-	+	+
	7		7	-	+	+	+
<i>Br. melitensis</i> biover suis	1		1	-	+	+	***
	2		2	-	-	+	-
	3		3	-	-	+	+
	4		4	-	-	+	(-)
	5		5	-	-	+	-
<i>Br. melitensis</i> biover ovis		<i>Br. ovis</i>		+	-	+	(+)
<i>Br. melitensis</i> biover canis		<i>Br. canis</i>		+	-	+	(+)
<i>Br. melitensis</i> Biover neotomae		<i>Br. neotomae</i>		-	+	-	-

* More differentiation of *brucella abortus* biovar 3 and six is by using thionine at 40mg/ml biovar 3= + and biovar 6= -

** Some strains are inhibited by basic fuchsin.

*** Some isolates are resistant to basic fuchsin.

(+) Most strains positive

(-) most strains negative

According to Bergy's manual (1984), *Br. melitensis* was further divided into three biovars, *Br. canis* have no biovars.

All species and biovars of *brucella* show more than 90% DNA homology. Polymorphism in some genes as identified by DNA technologies allows for differentiation (Cloeckaert *et al.*, 1996).

1.2.5 Economic importance and impact:

There is no doubt that *Br. melitensis* infection cause significant economic losses. Although the financial loss expressed in any currency may vary from one country to another.

The farmer suffers loss of income due to abortion, the consequent loss of milk production and a prolonged fattening time of lambs (meat production) due to birth of premature animals and low fertility rates (Chaukwa, 1987). Human brucellosis causes physical and psychological suffering due to infection, hospitalization, the cost of drugs and the loss of work or income due to illness.

The country incurs costs generated by prophylactic activities taken to control brucellosis i.e. vaccination by the veterinarians and their assistants, vaccine costs and compensation paid to the farmers for sanitary slaughter of infected animals. Consequently, control and eradication of *Br. melitensis* eventually pays off (Chaukwa, 1987).

1.2.6 Epidemiology:

Brucellosis in sheep and goats is usually caused by *Br. melitensis*. Infection with *Br. abortus* is rare.

The source of infection is an aborting animal (Cunningham, 1977).

As in cattle, the surroundings where lambs are born to infected ewes or where abortion takes place become greatly contaminated. Animals may contract brucellosis by oral or cutaneous routes, or at birth. Infection by inhalation is also possible when healthy and aborting animals are kept in overcrowded pens with poor sanitary measures (Stableforth and Galloway, 1959; Schnurrenberge *et al.*, 1975; Dekeiker, 1981; Peelman and Dekeyer, 1987).

Transmission of *Br. melitensis* from flock to flock usually follows the movement of infected pregnant females. However it can also occur via an infected male. Wild animals and dogs may transmit parts of aborted foeti to other areas (Alton, 1985; Mikolon *et al.*, 1998a).

The incubation period after infection varies from 15 days to several months depending on the invasion site and the infecting dose.

Therefore, it takes sometime for signs of infection to occur. In naturally infected sheep the only symptom noted is abortion. In infected goats abortion and sometimes also mastitis can be observed (Nabil, 2004) infected goats that do not abort give less milk than uninfected goats. Abortion usually occurs at 3-4 months into pregnancy, and in a susceptible flock it may reach epidemic proportions.

Goats that have aborted once are not likely to abort a second time. Sheep may abort a second time, as they can recover from the first infection. Both sheep and goats may shed *brucella* with any subsequent parturition (Enright, 1990) Retention of the placenta may or may not occur. It is also possible that infected pregnant goats that have been born

into an infected flock may give birth at the normal time (Stableforth and Galloway, 1959). Therefore, brucellosis in chronically infected flocks, becomes evident, only through infected people who have been in contact with infected animals or consumed their milk or cheese.

Both sheep and goats may show signs of lameness, hygroma and cough but the predilection sites of *B. melitensis* are the uterus, udder and the mammary lymph nodes in females and the testicles in males (Blood and Rodastitis, 1989 and Musa *et al.*, 1990). Strangely enough, interference with fertility caused by orchitis seems to be limited.

Infected sheep and goats may excrete *brucella* in the milk for years but sheep may also cause excretion during one or more lactation periods (Stableforth and Galloway, 1959; Alton, 1985; Enright 1990).

1.2.7 Resistant to infection:

Resistant to infection resembles *Br. abortus* infection in cattle. Age, sex and natural resistance to *brucella* may influence the progression of infection, sexually immature animals may show some resistant to infection whereas sexually mature animals are susceptible to infection, which in pregnant animals may result in abortion (Alton, 1985). Males are less susceptible to infection than females. There is very little difference between goat's breeds in their susceptibility to the *Br. melitensis* whereas breeds of sheep differ in their susceptibility, milking breeds of sheep seem to be more susceptible to *Br. melitensis* infection than sheep kept for meat production (Alton, 1985).

1.2.8 Survival of *brucella* in the environment:

Temperature, humidity and pH of the environment influence the survival of *Br. Melitensis* as well as that of *Br. abortus*.

Brucella are sensitive to direct sunlight, disinfectant and pasteurization. In dry conditions they survive only if embedded in protein. In optimal conditions *brucella* survive in tap water, damp soil, urine, aborted foeti, uterine exudate and in frozen tissues (Davies and Casey, 1973; Wray 1975).

1.2.9 Antibiotic sensitivity:

According to Bergey's Manual of systematic bacteriology (1984), nearly all *brucella* strain are sensitive in vitro to rifampicin, gentamicin and tetracycline. Most strains are also susceptible to erythromycin, ampicillin, chloramphenicol, kanamycin and combination of sulfa-methoxisole and trimethaprim.

Susceptibility to the antibiotics varies between species and even between biovars and strains of the same species.

Most *brucella* strains are resistant to polymyxin, penicillins, cephalosporins and nalidixic acid and nearly all strains are resistant to nystatin, linomycin, clindamycin, bacitracin and vancomycin.

1.3 Diagnosis of brucellosis:

Many methods are used for the diagnosis of brucellosis. The aim of brucellosis diagnosis is to identify and eliminate infected animals.

Control or eradication of brucellosis would not be a problem if an easy, rapid sensitive and highly specific test existed.

1.3.1 Direct smear:

Brucella organism can be seen in large numbers in films prepared from fresh samples of infected placenta, foetal stomach contents, vaginal swabs and ram semen after staining with modified zeihl-Neelsen stain. The organism stain pink against a blue background and appears single or in clumps intracellular as well as extracellular (Buxton and Fraser, 1977).

1.3.1.1 Bacteriological examination:

The isolation of the causative agent is the accurate method for diagnosis of brucellosis in animals and man. Isolation procedures are time consuming, laborious and costly and it is necessary to isolate and identify the organism for confirmation and epidemiological studies.

Several media are suitable for the isolation of *brucella*, such as, serum-Dextrose agar, serum tryptose agar, glycerol dextrose agar, *brucella* agar and potato agar. The use of selective media (Kuzdas and Morse, 1953) is necessary when isolation is attempted from grossly contaminated material. Solid media are preferred for the isolation and propagation of *brucella* because they facilitate recognition (Alton *et al.*, 1975).

The most suitable materials for isolation of the organism are foetal membranes, foetal stomach content, milk and ram semen. For *Br. abortus* cultures must be incubated in the presence of 5 to 10% CO₂ (Stableforth and Galloway, 1959).

1.3.1.2 Guinea pig inoculation:

Guinea pigs are susceptible to infection with *brucella* and are used for diagnostic purposes.

Animal tissue, secretions and excretions are inoculated intraperitoneal if the material is free from contamination. Milk or decomposed animal tissues are inoculated subcutaneously or intramuscularly. In the case

of milk a mixture of cream and sediment is used. Two animals are used for each test; one will be killed after three weeks and the other after six weeks. The animals are examined for lesions and the sera tested for agglutinins. Typical lesions include necrotic foci in spleen, liver, lymph nodes and orchitis in male guinea pigs.

The spleen, lymph node and other tissues containing lesions are minced and cultured on solid media as serum dextrose agar without inhibitory dyes or antibiotics.

A positive serum agglutination test without positive culture is enough to justify a diagnosis of brucellosis (Alton *et al.*, 1975).

1.3.1.3 Serological Tests:

A definite diagnosis of *brucella* infection is obtained by isolation and identification of the causal agent but it is not always possible to isolate the organism from infected animals.

A variety of serological tests are therefore extensively used for routine diagnosis of the disease. However, it is believed that no single method is completely satisfactory because none of the tests is reliable for detecting infected animals in the incubation period. (Buxton and Fraser, 1977).

According to Alton (1987) serological tests for the diagnosis of *Br. melitensis* infection in small ruminants are not reliable as those for *Br. abortus* infection in cattle.

Many workers used several serological tests for the diagnosis of brucellosis in goats and compared the sensitivity and specificity of the tests.

Falade (1978) used the Tube Agglutination Test (TAT), Rose Bengal Plate Test (RBPT) and the Milk Ring Tests (MRT) for the diagnosis of caprine brucellosis in Nigeria and reported that the Serum Agglutination Test (SAT) was a better diagnostic tool in the area.

Waghela *et al.* (1980) compared four serological tests for diagnosis of caprine brucellosis; these were TAT, RBPT, Complement Fixation Test (CFT) and Agar Gel Plate Test (AGPT)

The sera tested were obtained from farms infected with *Br. melitensis*. The results showed that 92 goats were negative and 29 positive to all four tests. Sera from 85 goats were positive to one or more tests. The result also showed that RBPT was the most sensitive test and AGPT the most specific, it was also suggested that the TAT adds little information when used with other tests and the RBPT and AGPT are useful for testing caprine brucellosis when facilities for the CFT were not available.

Blasco *et al.* (1994). Found that the CFT was less sensitive than RBPT when testing culturally positive sheep.

Diaz Aparicio *et al.* (1994), employed five tests for the diagnosis of brucellosis in goats. The tests included RBPT, CFT, Enzyme-linked Immunosorbent assay (ELISA). Radial Immunodiffusion (RID) and Counter-Immunoelectrophoresis (CIEP), they found that the sensitivity was

100% for (RBPT) 94% for (CFT) and ELISA and 93% for RID. All tests were 100% specific because they gave negative results. When testing sera from *brucella* – free goat. Mikolon *et al.* (1998) employed many tests for the diagnosis of caprine brucellosis and reported that RBPT was a good test because of high sensitivity at week 24 post infection in addition to ease of performance and low cost.

1.3.1.4 Rose Bengal Plate Test (RBPT):

The use of RBPT which is easy to perform and is considered a valuable screening test to detect the presence of *Br. abortus* infection in cattle (Farina, 1985) also it can be used as a definitive test (Nicoletti, 1967). Rose and Roepke (1957) modified the plate agglutination test by buffering the antigen at pH (4) immediately before use to differentiate specific *brucella* agglutinins from the non-specific factors.

However, recently it was found to detect IgG1 and IgM isotypes in bovine, sheep and goat sera and diagnosed the acute and chronic forms of the disease (WHO, 1993).

The test is less effective than the CFT at detecting brucellosis in individual sheep and goats (FAO, WHO, 1986).

Furthermore, its efficacy is influenced by the cell concentration and the standardization procedure of the antigen. (Hosie *et al.*, 1985; Blasco *et al.*, 1994a).

Sera negative for RBPT are not tested further but positive ones are tested by SAT and CFT (Morgan *et al.*, 1978). Nevertheless false negative reactions have been obtained (Morgan, 1971; Miller *et al.*, 1973, Lapraik *et al.*; 1975 and Belkin, 1977).

1.3.1.5 The Tube Agglutination Test (TAT):

The test is widely used for the diagnosis of brucellosis of animals and man. It is the method of choice for cattle.

However, many infected goats, sheep and human beings do not give a positive agglutination test despite, the fact that they may be positive to other tests such as the CFT (Stableforth and Galloway, 1959). Application of the agglutination test led to the recognition of brucellosis of goats (Zammit, 1905).

The TAT sometimes give a false positive reaction as a result of cross-reaction between antigens of *brucella* and unrelated organism such as *Yerseina enterocolitica* or they may be due to non-specific agglutinins distinct from antibodies, which are present in certain bovine sera (Hess, 1953a, b).

It was reported that the traditional agglutination test with sheep and goats sera lacks both sensitivity and specificity even when 5% saline solution which- improve the performance of the test is used.

1.3.1.6 The CFT:

The CFT is considered to be the most effective test for diagnosis of brucellosis in small ruminants (FAO, WHO, 1986). It is used as confirmation of RBPT and TAT. The test was superior to other test in sensitivity and specificity in cattle (Morgan *et al.*, 1973).

Sera from small ruminants may show anti-complementary activity in the CFT. Although the anti-complementary activity can be eliminated when the sera are inactivated for 55 minutes at 60°C (Bercovich, unpublished data). The test remains tedious to perform.

Moreover, acutely or chronically infected animals as well as latent carrier may elude detection with the CFT (Karmann and Schloz, 1956; Farina, 1985; Blasco *et al.*, 1994b).

1.3.1.7 AGPT:

The test was described by Bruce and Jones (1958). They found that cultures of *Br. melitensis* but not *Br. abortus* and *Br. suis* produced a diffusible antigen which formed one to three precipitation bands with sera of rabbits, goats and cattle infected with *Br. abortus* and *Br. melitensis*. Waghella *et al.* (1980) reported that the AGPT was a very specific test.

1.3.1.8 ELISA

Studies were conducted to choose a reliable diagnostic procedure by comparing serological test with various ELISA procedures.

Most studies agree that the ELISA is as specific as the CFT but it is more sensitive. Yet, for a reliable diagnosis of infected animals studies suggest using the ELISA in combination with other tests (Bercovich *et al.*, 1998; Jacques *et al.*, 1998; Mikolon *et al.*, 1998b). Other studies consider the ELISA suitable for screening flocks of sheep and goats for brucellosis (Biancifion *et al.*, 1996; Sting and Orthmann, 2000). Nevertheless small ruminants should be tested with the ELISA, CFT to prevent the spread of brucellosis after an out break of the disease in an area with low prevalence of brucellosis or in an area free from brucellosis (Bercovich *et al.*, 1998).

1.3.1.9 Polymerase Chain Reaction (PCR :)

The technique is a very useful tool for the diagnosis of brucellosis

because of its simplicity; high degree of sensitivity and specificity together with its speed, versatility in sample handling and risk reduction for laboratory personnel. (Morta *et al.*, 2001).

Serum samples should be used preferentially over whole blood for the molecular diagnosis of brucellosis (Zerva *et al.*, 2001).

The test was used to diagnose brucellosis in goat and it was shown to be more sensitive than the RBPT and culture techniques (Leal. Klevezas *et al.*, 2000).

1.3.2 The Milk Ring Test (MRT):

The test widely used to detect brucellosis in dairy cattle is not sensitive enough to detect brucellosis in sheep (Shimi and Tabatabai; 1981). However, because the test is simple and easy to perform it might be useful to detect *brucella* antibodies in milk from dairy sheep and goats kept for cheese production. The antigen is a suspension of the organisms stained with haematoxylin (blue colour) or tetrazolium (red colour), many workers prefer the tetrazolium-stained antigen for testing sheep and goat milk.

The MRT using 8 ml milk (Bercovich and Lagendijk, 1978) or the MRT performed on three parts sheep milk supplemented with one part pooled cows milk which tests negative for *brucella* with the MRT strongly increases the sensitivity of the test (Bercovich, unpublished data).

1.3.3 Whey Agglutination Test (WAT):

The test is of value for detecting animals which are excreting *Br. abortus*. After preparation, whey is tested by the same method as the TAT (Buxton and Fraser, 1977).

1.3.4 Capillary Stained Antigen Test (CSAT):

This test was used to detect antibodies to *brucella* in bovine milk by King (1951). He found that the test was satisfactory and was not affected by low fat content.

1.4 Disease prevention and control:

Effective control of brucellosis largely depends on the co-operation of the flock owner (Robertson, 1976).

At the farm level good hygiene, management and vaccination are necessary. Treatment of infected sheep and goats with antibiotics is not done because the antibiotics may appear in the human food chain and this would be disastrous for the cheese production industry.

Instead, efforts are directed towards controlling and eradicating brucellosis from small ruminants. Serological testing and slaughter of the animals that react positively with *brucella* antigens successfully eradicated brucellosis in several countries (FAO, WHO expert committee on Brucellosis, 1953). This procedure, however, is not easy to apply in developing countries where usually animals are not tagged. In areas with endemic brucellosis only vaccination against *Br. melitensis* may reduce the number of infected flocks and eventually permit brucellosis control (Plommet; 1986).

Currently two vaccines are in use the H38 and Rev1. The H38 vaccine is composed of killed, smooth, virulent cells of *Br. melitensis* in adjuvant. The vaccine gives good protection and can be administered to pregnant and lactating animals (Alton, 1985; Plommet, 1991). The Rev 1 vaccine is composed of living attenuated cells of *Br. melitensis* and is used in most countries that vaccinate small ruminants against *Br. melitensis* (Blasco, 1997). Although vaccination with $1-2 \times 10^9$ CFu (classical dose) of 4-6 months, old, or at non- pregnant adults protects the animals for several years, the vaccine also has some disadvantages since the vaccine consist of living *Br. melitensis* cells it may cause abortion in pregnant sheep and goat and it is excreted in the milk (Hagan and Bruner, 1988).

While a year after vaccination most CFT results are negative, the response to the vaccination may last long than 24 months (Gaumont *et al.*, 1984). To limit the risk of abortion and excretion of brucella following the vaccination, the conjunctival vaccination with 5×10^4 CFu was introduced. Conjunctival vaccination with a reduce dose, is not only safer it is also easier to apply (Alton, 1985; Plommet, 1991).

Rev 1 vaccine is an attenuated *brucella* strain that is dangerous for man (Elberg, 1996).

There is another type of vaccine that is strain 19. Strain 19 has not

given good result in goats infected as kids or adults before service (Stableforth and Galloway, 1959).

1.5 Brucellosis in the Sudan:

1.5.1 Human brucellosis:

Human brucellosis was diagnosed in the Sudan as early as 1904 in a patient at Barber in the Northern Province (Haseeb, 1950) four years later; Simpson (1908) reported 20 cases for Malta fever clinically diagnosed in man in Kassala and Blue Nile Provinces.

The disease was diagnosed in all provinces except Bahar Elgazal up to 1955 (Haseeb, 1950, Daffalla; 1962). Since then the occurrence of the disease was regularly mentioned in the reports of the Sudan Medical Services.

1.5.1.1 Bovine Brucellosis:

The disease was first diagnosed by Bennett (1943). Who isolated *Br. abortus* from an aborted foetus of a cow in a dairy farm near Khartoum. After the diagnosis of the disease by Bennett (1943) extensive serological surveys were done to detect antibodies of *brucella* in cattle, sheep, goats and camels in most parts of the country.

In the Gezira area in 1953, the cows supplying the milk were examined for antibodies to *brucella* as the result of several cases of undulant fever among European residents in that area, many cows gave positive reaction to the SAT and *Brucella melitensis* was isolated from the milk of one of them.

Br. melitensis was also isolated from the milk of an ewe a sheep and

a goat sharing grazing with cattle (Dafalla and Khan, 1953).

The disease was serologically diagnosed in dairy herds in Malakal and Tong in the Southern Sudan in 1953, in Elobeid dairy in western Sudan and in Kenana cattle at Singa in the Blue Nile province (Daffala and Khan, 1958).

The disease was also serologically diagnosed in the upper Nile Province in the Southern Sudan by Nasri in 1960.

Serological diagnosis of the disease was also done in various parts of the country by other workers (Abdulla, 1966; Mustafa and Hassan, 1969; Ibrahim and Habibella, 1975; Habibella, 1977; Omer *et al.*, 1977, Bakhiet, 1981; Shallali *et al.*, 1982; Elwali *et al.*, 1983; Suliman, 1987; El Hussein *et al.*, 1991; Mohmoud, 1995, Musa, 1995, Hayfa, 2001 and Rias, 2004)

1.5.1.2 Brucellosis in camels:

The few serological investigations which were conducted revealed the presence of antibodies to *Br. abortus* in some of the tested camels (Mustafa and El Karrim, 1971; Abu Damir *et al.*, 1984, Fayza *et al.*, 1990, Musa, 1995, Mozamil, 2002 and Ahmed 2004).

1.5.1.3 Caprine Brucellosis:

Brucellosis in sheep and goats is mainly caused by *Br. melitensis* which is the most virulent species of all of the *brucella* (OIE, 1996). Infection with *Br. abortus* is rare (Nicoletti, 1980)

Some work was done on bovine brucellosis but little attention was given to caprine brucellosis, despite the fact that the veterinary services became aware of caprine before bovine brucellosis.

Some workers who carried out serological investigation on the prevalence of brucellosis in cattle also tested at the same time available sheep and goats but the numbers tested were much less than cattle. The rates of positive in goats were 2.5% - 5.9% in Gezira (Daffalla and Khan, 1958), 5.7% - 8.3 % in the Upper Nile Province (Nasri, 1962), 1.5% in Wadi Halfa (Abdullah, 1966). El Sawi *et al.*, (1981) reported 2.2% from different provinces. (Fayza *et al.*, 1990) 1.0% in Khartoum (Osman and Adlan, 1987; Ginawi 0.0% 1997), 2.5% in Khartoum by Hayfa (2001) and 3% in Darfur state by Rias (2004).

1.6 Isolation of *brucella* in Sudan:

Br. abortus was isolated from aborted bovine foeti (Bennett, 1943; Daffalla and Khan, 1958; Musa and Mitchell, 1985; Khalafalla *et al.*, 1987 and Musa *et al.*, 1990).

The organism was also isolated from synovial fluid of cattle by Shigidi and Razig (1973), from bovine milk (Ibrahim, 1975; Khalafalla *et al.*, 1987; Suliman, 1987 and Musa, 1995). From camels in Butana area (Agab *et al.*, 1995), and from the blood of human patients in Khartoum (Erwa, 1958)

Br. melitensis was isolated from the milk of cattle, sheep and goats (Daffalla and Khan, 1958) and from a ram in an infected flock (Musa, 1995).

According to Musa (1995) the strains of *Br. abortus* isolated in the Sudan were typed as *Br. abortus* biovar 6 and those of *Br. melitensis* as *Br. melitensis* biovar 3.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Samples for serological examination:

2.1.1 Source of samples:

Samples consisting of serum and milk were collected from different breed of goats and different ages in different localities in Omdurman area. (Table 3)

Serum and milk samples were examined for the presence of antibodies to *brucella*.

2.1.2 Collection of samples

2.1.2.1 Milk samples:

After examining goats for udder and teat abnormalities milk samples were collected from healthy animals.

The whole udder was washed, dried and the tip of the teat was disinfected with 70% alcohol. The first stream of milk was discarded and then five ml of fore milk from each half of the udder were taken directly into a labeled sterile universal bottle and placed on ice in a thermos flask (Alton *et al.*, 1988).

2.1.2.2 Serum samples:

Five ml of blood were collected in sterile tubes from the jugular vein using a disposable syringe after clipping the hair and disinfecting the area with methyl alcohol.

Table (3): Source and number of samples collected from Omdurman area.

Area of collection	No. of samples	Type samples	
		Serum	Milk
1. Ombada	29	29	26
2. Alsarha	10	10	8
3. Alsug alshaby	11	11	5
4. Hay Al Arab	20	20	16
5. Elthora	16	16	12
6. Wad Albkheet	30	30	26
7. Gabal Toria	48	48	45
Total	164	164	138

The syringes were placed in a slanting position and after collecting were taken to the laboratory on ice and placed in the refrigerator over night. Then the serum was collected in bijoux bottles. The sera were tested immediately after collection or kept at -20°C until used within 48 hours (Alton *et al.*, 1988).

2.2 Serological tests:

The RBPT, AGPT and capillary tube agglutination tests (CTAT) were used to determine the presence of antibodies in sera.

2.2.1 RBPT:

2.2.1.1 Antigen for the test:

The antigen used in the RBPT was obtained from C.V.L, Soba. The antigen was prepared and standardized as described by Alton *et al.*, (1988).

2.2.1.2 Procedure of the test:

The serum samples and the antigen were removed from the refrigerator and placed at room temperature for one hour.

According to Alton *et al.* (1988), equal volumes of undiluted serum and stained antigen were placed on a slide, mixed well with glass rod, rocked gently for 4 minutes and then the test was read.

Any degree of agglutination was a positive result, while no agglutination was regarded as negative result.

2.2.2 CTAT:

2.2.2.1 Antigen for the test:

The antigen used for the RBPT was used in this test.

2.2.2.2 Procedure of the test:

The test was done as described by Luoto (1953). Approximately, one third of the capillary tube was filled with the stained antigen and the reminder with undiluted serum by means of capillary action. The tubes were placed in a vertical position in wax with the antigen at the bottom. The tubes were then incubated at 37°C for 2 hours.

The macroscopic agglomerates indicated a positive reaction absence of agglomerates, indicated negative reaction.

2.2.3 AGPT:

The test was carried out as described by Nasri (1967).

2.2.3.1 Preparation of The antigen:

A suspension of *Br. melitensis* antigen obtained from plasmated laboratory products, United Kingdom, was centrifuged at 10.000 r.p.m for ten minutes. The pellet was removed, resuspended in saline and centrifuge again in the same manner. This was repeated twice. The final pellet was suspended in distilled water and the organism were disrupted by three rapid cycles of freezing and thawing by alternating in the deep freeze at -20°C and warm water at 56°C.

2.2.3.2 Preparation of Agar Gel:

The agar gel was prepared by dissolving 1.4 grams of purified agar (OXOID) in 100ml of normal saline- 0.2 mg of sodium azide was added as a preservative. The gel was distributed in 12ml amounts in plates, and after solidifying at room temperature was kept in the refrigerator until used.

2.2.3.3 Method of testing and examination of Agar plates:

A rosette of six peripheral wells and a central well were cut with a template. The plugs were removed with a Pasteur pipette.

The distance between the central and peripheral wells was 0.5 cm. Each peripheral well was carefully filled with serum to be tested while the central well was filled with antigen. The plates were incubated for ten days at room temperature in a humid chamber and examined daily for precipitation bands in a dark room through transmitted light.

2.3 Test For Detecting Antibodies In Milk:

2.3.1 MRT:

2.3.1.1 Antigen for the test:

Stained antigen supplied by the C.V.L, Soba.

2.3.1.2 Procedure of the test:

The procedure followed was described by Alton (1988).

- The milk samples were shaken gently to disperse the cream
- One ml of milk was pipetted into an agglutination tube.
- One drop of antigen (0.03ml) was added by a dropper.
- Then mixed gently and incubated at 37°C for three hours.

Results were recorded as follows:

- If agglutinated antigen falls to the bottom of the tube leaving the milk column white, this indicates a positive result.
- Ring formation at the top indicates a positive reaction.
- A clump of agglutinated antigen dispersed in the milk column is also a positive result.
- When no change in the appearance of the milk column occurs this means a negative result (Alton *et al.*, 1988).

CHAPTER THREE

RESULTS

3.1 Serological tests:

3.1.1 RBPT:

Out of the 164 samples tested according to the area s, the results were Ombda 22 (13.5%), together with Sarha and wad Albkhaet 5 (3.07%) respectively (Table 4). All of the samples showed granular agglutination clearly visible by the naked eye.

3.1.2 CTAT:

Twenty seven (16.57) sera were found positive by this test (Table 4). In positive tests, macroscopic agglomerates readily visible to the naked eye appeared in the capillary tube.

Negative reaction was indicated by the absence of such particles.

3.1.3 AGPT:

Precipitation band appeared after 2-3 days with each of the positive sera, but the negative serum gave no band. Twelve (12) positive sera were tested by RBPT and CTAT control with another (12) negative control sera.

All tested positive sera gave positive results and negative sera showed negative results.

3.2.1 MRT:

Thirteen (9.28%) samples were positive to the test. In ten samples the antigen was clumped at the bottom of the tube. Ring formation at the top of the milk column was seen in the other samples.

3.3 Distribution of positive reactors according to the serological test among the different localities:

The distribution of positive reactors to the different serological test, among the various localities showed little differences. However, slightly more reactors were found in Ombada, while no positive reactors were detected by any of the three tests in Althora and Gabl Toria.

Table (4): The results of Brucellosis survey in goats in Omdurman area

Test		A	B	C	D	E
RBPT	+ve	25 (13.5%)	5 (3.07%)	-	-	-
	-ve	6 (3.68%)	35 (21.47%)	31 (19.01 %)	16 (9.82%)	48 (27.44%)
CTAT	+ve	22(13.5%)	5 (3.07%)			
	-ve	6(3.68%)	35 (12.47%)	31 (19.01%)	16 (9.82%)	48 (29.44%)
AGPT	+ve	12 (50%)	-	-	-	-
	-ve	-	-	8(33.33%)	-	4(16.67%)
MRT	+ve	5 (3.57%)	4 (2.86%)	3 (2.14%)	-	1 (0.71%)
	-ve	21 (15%)	29 (20.71%)	22 (5.71%)	12 (8.57%)	43(30.71%)

A= Ombda

B = Sarha + Wad Albkaet

C= Sug Shaby + Hay Alarab

D= Althora

E= Gabl Toria

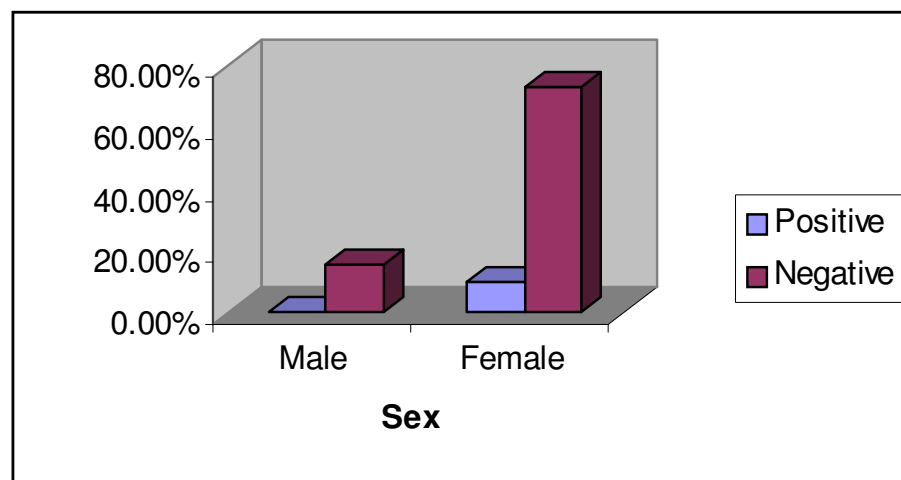
Table (5): Agreement between tests (Kappa statistic):

Test	Agreement	Kappa statistic
RBPT and AGPT	50 %	1*
RBPT and CSAT	72.36%	1*
RBPT and MRT	75.59%	0.39 **
AGPT and CSAT	50%	1*
AGPT and MRT	47.11%	0.31*
CSAT and MRT	75.59 %	0.39**

* = complete agreements.

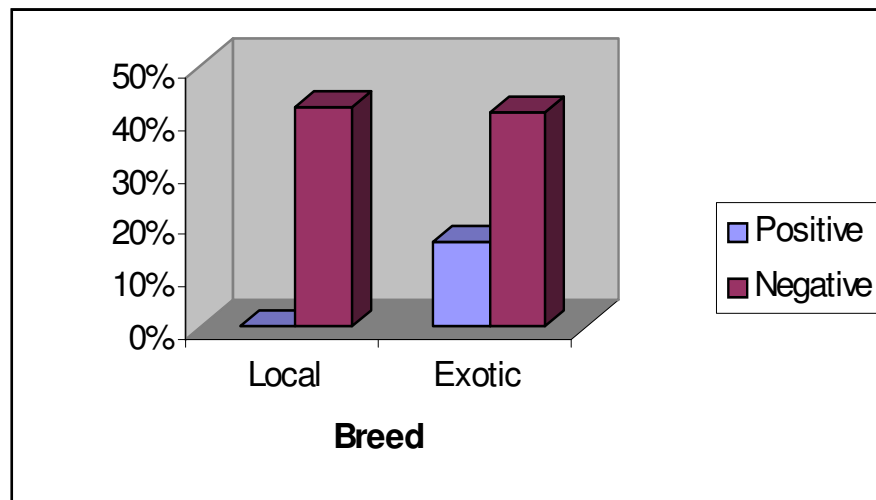
** = poor agreement

Figure 1: The relationship between sex and presence of brucellosis in goats based on Rose Bengal Plate Test



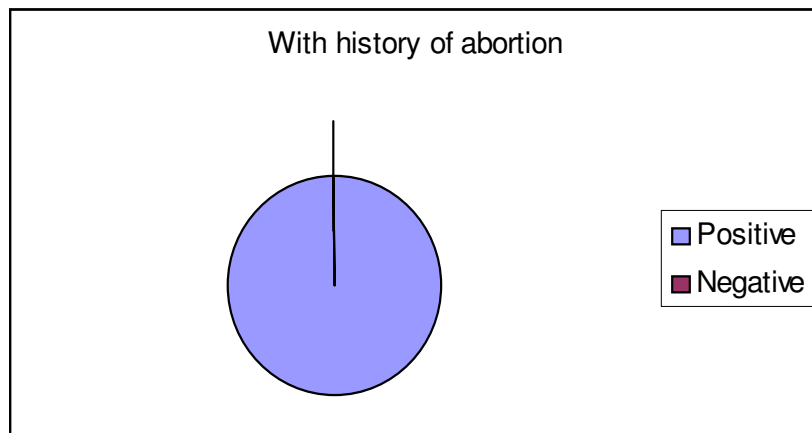
Chi-square (χ^2) = 1.774 P -value = 0.183 (not significant)

Figure 2: The relationship between breed and presence of brucellosis in goats based on Rose Bengal Plate Test



Chi-square (χ^2) = 23.754 P -value = 0.000 (highly significant)

Figure 3: The relationship between history of abortion and presence of brucellosis in goats based on Rose Bengal Plate Test



Chi-square (χ^2) = 4.609 P -value = 0.032 (significant)

CHAPTER FOUR

DISCUSSION

The Sudan has 43.8 million goats. These animals have a great economic importance and are kept for meat, milk, hair and skin.

Goats are kept by individual owners in small groups. During the day the goats are brought together to graze by a shepherd and in the afternoon they dispersed to return to their owners. In this type of management goats come in contact with each other for short periods under dry environmental conditions and high temperature.

Caprine brucellosis in the country was reported for the first time by Bennet (1943).

However serious attempts to study the disease in goats were not made until 1953 as a result of a serious outbreak of undulant fever among European residents in Barkat in the Gezira (Daffalla and Khan, 1958).

During the present work three serological tests, RBPT, CTAT and AGPT were used and milk samples were examined by the MRT.

The result of the study showed, prevalence rate of (16.57%) by the RBPT, (16.57%) by the CTAT.

The result of milk samples showed prevalence rate of (9.28%) by the MRT.

The prevalence (16.57%) obtained by the RBPT during present work is higher than the result reported by earlier workers.

El Sawi *et al.* (1981) found that 0.65% of goats tested were positive, fayza *et al.* (1990) examined 2233 sera from goats destined for export and found that only 0.1% were positive, Ginawi (1997) screened 190 goats sera and found them all negative (0%), and Hayfa *et al.* (2001) examined 1000 sera from goats and found that only 1.5% were positive.

It is difficult to explain these differences in the results obtained by RBPT. However, the goats tested were in different parts of the country and from different numbers and breeds, and this might have an effect on the result.

The finding of this test in the present work indicated that caprine brucellosis became a common and serious disease among the goats in Omdurman area.

The CTAT, for serum, during the present work showed that (16.57%) of the goats were positive reactors and this is the same as the result of the RBPT.

The prevalence rate. (16.57%) obtained by the CTAT during the present work is higher than the results reported by earlier workers.

Rias (2004) found that (0.3%) of the goats tested were positive.

The AGPT was found satisfactory by Waghela (1978, 1980). White (1958) described an agar gel diffusion test which was used for the diagnosis of contagious bovine pleuro pneumonia.

The test can be used to detect both antibody in the sera of animals and antigen in tissue fluids such as pleural exudates. Nasri (1967) used the test to diagnosis contagious bovine pleuro pneumonia.

A trial was therefore, made by testing (12) sera that is positive in the RBPT and CTAT together with (12) negative controls.

The results were encouraging because each of the positive serum gave one precipitation band. All the bands joined identically. They appeared after 2-3 days and were faint which may be due to a poor quality of the antigen.

The percentage of the positive reactors in milk was found to be (9.28%). This rate is higher than the result reported by the other workers.

There is only one record of testing goat milk by the MRT in the Sudan (Dafalla, 1962). Out of 138 milk samples 13 (9.28%) were found positive.

The results of the present work showed that the prevalence rate of caprine brucellosis in Omdurman area is slightly high; that might be due to the differences between localities, and increased numbers of exotic breeds and the poor hygiene in the area; also the type of management could affect the result, because at day time the goats graze together which will lead to that; healthy goats can take the infection from the diseased one through oral route by licking the aborted fetuses and the genital discharges of aborted females, or through ingestion of contaminated fodder and drinking water. Also in breeding time the females could get the infection from the carrier male, so these may explain why the percentage is slightly high.

The goats are considered as the major source of brucellosis in human. Mainly the disease is common among veterinarians, butchers farmers, and goats milk consumers. The poor hygiene of the farms; dirty workers, unsterilized and uncleaned equipments all these could increase the prevalence of the occurrence of the disease.

Eventually, this result might be attributed to the small number of the samples compared with the number of the samples of the other workers.

As the results showed a slightly increased percentage of the disease; I recommended that other researches keep searching in this area to face the progression of the disease.

Early detection and diagnosis of the disease by providing a necessary equipment for diagnosis will facilitate the control program of the disease and also by providing mobile clinics we could advice the farmers to separate unhealthy goats from healthy ones.

Lastly all these control measures could be achieved by a good health orientation through massmedia and local posters directed to those people concerned.

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